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β-Amyloid-Induced Cytotoxicity, Peroxide Generation and Blockade of Glutamate Uptake in Cultured Astrocytes

Maria L. de Ceballos[1], Begoña Brera[1] and María Paz Fernández-Tomé[2]

[1] Cajal Institute, CSIC, Madrid, Spain
[2] Institute of Pharmacology and Toxicology, CSIC, Faculty of Medicine, Complutense University, Spain

β-Amyloid (βA) is cytotoxic to neurons in culture by increasing hydrogen peroxide and altering calcium homeostasis. We have evaluated βA-induced cytotoxicity, peroxide generation and glutamate (Glu) uptake in cultured astrocytes. Twenty-four hours after a single addition of either βA_{25-35} or βA_{1-40} there was a concentration-dependentdecrease in viability. Catalase or vitamin E showed no protective effect against βA_{25-35}. Dithiothreitol (DTT), N-acetylcysteine (NAC) and cyclosporine A significantly prevented the toxic effects of both βA_{25-35} and peroxide, while inhibition of peroxide detoxifying enzymes enhanced toxicity. Exposure to βA_{25-35} or βA_{1-40} increased peroxides at 2 h and 24 h, which was prevented by DTT and NAC, but not vitamin E. βA_{25-35} inhibited Glu uptake in astrocytes and neurons in culture. Following exposure of neurons to βA for 24 h there was decreased uptake and increased Glu levels in the culture medium, that resulted in gradual excitotoxicity.

Key words: Alzheimer's disease; β-Amyloid; Glutamate; Peroxides.

Abbreviations: AD, Alzheimer's disease; βA, β-Amyloid; CNS, central nervous system; DHK, dihydrokainic acid; DTT, dithiothreitol; Glu, glutamate; MTT, 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide; NAC, N-acetylcysteine; SCR, scrambled peptide.

β-Amyloid (βA) is the major component of senile plaques and vascular deposits present in Alzheimer's disease (AD). This 39-42 amino acid peptide is cleaved from the amyloid precursor protein and several lines of evidence indicate that βA plays a crucial role in AD pathogenesis (1). βA is cytotoxic to neurons and clonal cell lines in culture by increasing hydrogen peroxide and altering calcium homeostasis (2, 3).

Astrocytes are crucial for normal central nervous system (CNS) function, providing ion homeostasis and metabolic substrates, controlling extracellular concentrations of glutamate (Glu), synthesizing neurotrophic factors, and finally, protecting neurons from stress. We have evaluated the cytotoxicity of βA peptides (βA_{25-35} and βA_{1-40}; 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay) and generation of hydrogen peroxide (dichlorofluorescein fluorescence) on cortical cultured astrocytes. Since Glu transport is sensitive to oxidants (4) it was also studied in primary neuronal and astrocyte cultures (^{3}H-Glu uptake).

βA_{25-35} or βA_{1-40} decreased viability in a concentration-dependent manner (E_{max50} around 0.5 μM). A subpopulation of astrocytes resistant to βA_{25-35} effects was also insensitive to peroxide. Catalase or vitamin E showed no protective effect against βA_{25-35} toxicity. Dithiothreitol (DTT, 2 mM), N-acetylcysteine (NAC, 5 mM) and cyclosporine A (0.5 mM) significantly prevented the toxic effects of both βA_{25-35} and peroxide (Figure 1). Inhibition of peroxide detoxifying enzymes, such as catalase and glutathione peroxidase, increased βA_{25-35} and peroxide toxicity. Exposure to βA_{25-35} or βA_{1-40} increased peroxides at 2 h and 24 h, which was prevented by DTT and NAC, but not by vitamin E (0.2 mM). Therefore, we demonstrate that peroxide generation is an early event in βA toxicity and not a consequence of ongoing cell death. These results suggest that βA-induced toxicity to astrocytes is, as in neurons, mediated by generation of hydrogen peroxide (5).

The effect of βA on Glu uptake was studied along that of dihydrokainic acid (DHK), prototype of astrocyte Glu transport inhibitors. βA was more potent than DHK in inhibiting Glu uptake and the effect of both was more marked on astrocytes than on neurons (Figure 2). Fol-

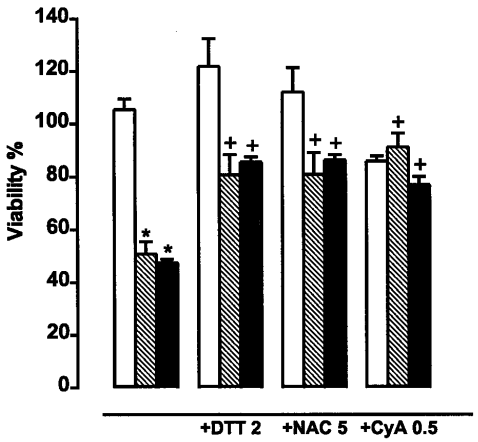

Fig. 1 Interference with free radical formation prevented βA and peroxide toxicity. Astrocyte cultures were exposed to the drugs and viability measured at 24 h. Open columns: scrambled peptide (SCR), hatched columns: βA_{25-35}, cross-hatched columns: hydrogen peroxide. Results are mean±SEM of two experiments done in quintuplicate. *p<0.05 different *vs.* untreated cultures or SCR; +p<0.05 different *vs.* single treatments (Student´s t test).

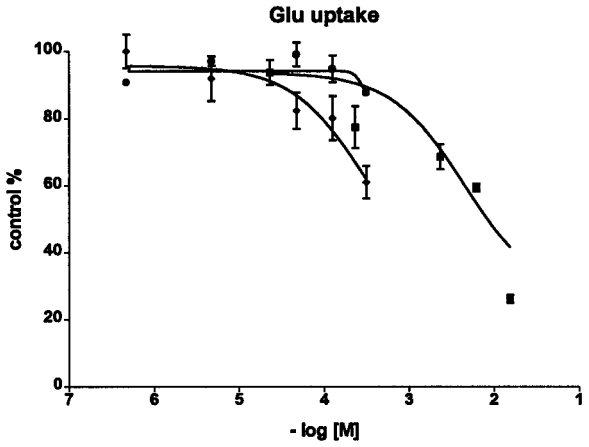

Fig. 2 βA_{25-35} inhibited ^{3}H-Glu uptake into neurons in culture. SCR: circles; βA: diamonds; DHK: squares. Results are from three experiments done in quintuplicate.

lowing exposure to βA for 24 h there was decreased uptake and increased Glu levels in the culture medium. Interestingly, βA gradually (2–3 days after βA addition) induced toxicity in neuronal cultures, while DHK, which at the highest concentration tested slightly increased Glu levels in the medium, was not toxic. In agreement with previous work (6) we have shown that βA inhibits Glu uptake in astrocytes but, more importantly, we here demonstrate that it inhibits the uptake in neurons as well. Furthermore, this results in Glu excitotoxic levels that may be involved in the gradual toxicity of βA to neurons in culture. In summary, the effects of βA on astrocytes may play an important role in the neuronal function and survival and may be relevant to AD pathology where oxidative stress has been demonstrated.

Acknowledgements

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References

1. Selkoe DJ. The molecular pathology of Alzheimer's disease. Neuron 1991; 6:487–98.
2. Behl C, Davies JB, Lesley R, Schubert D. Hydrogen peroxide mediates amyloid β protein toxicity. Cell 1994; 77:817–27.
3. Mattson MP, Cheng B, Davis D, Bryant K, Leieburg I, Rydel R. β-Amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. J Neurosci 1992; 12:376–89.
4. Trotti D, Danbolt NC, Volterra A. Glutamate transporters are oxidant-vulnerable: a molecular link between oxidative and excitotoxic neuro- degeneration. Trends Pharmacol Sci 1998; 19:328–34.
5. Brera B, Serrano A, de Ceballos ML. β-Amyloid peptides are cytotoxic to astrocytes in culture: a role for oxidative stress. Neurobiol Dis 2000; 7:395–405.
6. Harris.ME, Wang Y, Pedigo Jr NW, Hensley K, Butterfield DA, Carney JM. Amyloid β peptide (25–35) inhibits Na^{+}-dependent Glutamate uptake in rat hippocampal astrocyte cultures. J Neurochem 1996; 67:277–86.

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Corresponding author: Dr. Maria L.de Ceballos,
Cajal Institute, CSIC, Doctor Arce, 37, 28002 Madrid, Spain
Tel:+34 91 5854716, Fax: +34 91 5854754
E-mail: mceballos@cajal.csic.es